Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 17(66) No. 1 – 2024
https://doi.org/10.31926/but.fwiafe.2024.17.66.1.11

THE PHYTOCHEMICAL QUANTIFICATION AND PHARMACEUTICAL POTENTIAL EVALUATION OF DIFFERENT SOLVENT EXTRACTS OF *SESBANIA ACULEATA*

Nikhil MEHTA[1] Priyanka RAO[1]
Ritu BADAL[1] Raman SAINI[1]

Abstract: *The present study aims for screening phytochemical content and exploring antibacterial, antioxidant and anticancer potential of different solvent extracts of different parts of Sesbania aculeata. The highest extract yield obtained for the ethanolic extract with the highest amount of the phytochemicals (alkaloids, saponins, tannins, phenols and flavonoids) tested. Ethanolic extracts (20.8-31.1 mm zone of inhibition) showed best antibacterial activity against the tested bacteria and also the highest antioxidant activity tested by the 2,2-diphényl-1-picrylhydrazyl free radical scavenging activity and ferric reducing activity. The anticancer activity of ethanolic extracts tested against human oral cancer cell line SSC-29B and human kidney cancer cell line 786-O, where only seed coat extract which showed anticancer potential while other extracts showed negligible activity.*

Key words: *Antibacterial; Anticancer; Antioxidant; Phytochemicals.*

1. Introduction

Plants are the most valuable gift from nature, as they are not only source of food, but also possess various other properties that include treatment of various diseases. Phytochemicals or secondary metabolites that are responsible for the plant characters like colour, flavour and disease resistance, also contribute to other properties exploited by the humans. These phytochemicals like alkaloids, flavonoids, terpenoids, tannins, phenolic acids, etheric oils, vitamins, hormones, amino acids and amines etc. are responsible for different functions like defence against microbes and pests, formation of hormones and pigments etc. under different environment conditions. Various parts of the plants have been used by the primitive man for the treatment of diseases. In the modern time with the advancement of

[1] Department of Biotechnology, Kurukshetra University, Kurukshetra, Haryana, India.
Correspondence: Raman Saini; email: ramanksaini@rediffmail.com.

technology, the plant's active ingredients are used in various forms like powder or extract or their derivatives. Despite the ample acceptability of the phytomedicines, their uses are restricted due to lack of studies on efficacy and validation of such medicines. So there is urgency for the validation of the activity of such compounds that are responsible for curing various ailments as well as other diseases. Legumes seem to be a good possible target for the study, because the members of this family make a major contribution to the diet of people along with many possessing health benefitting properties. Of the various legume plant species, *Sesbania aculeata* is used for the present study. *Sesbania aculeata* is a multipurpose legume crop plant of the Fabaceae family and is native to India, Pakistan and other Asian particularly South Asian countries [3]. It is cultivated mainly for soil enrichment, fibre, fuel wood, fodder, paper, the dye industry and other agro-forestry uses but it also possesses various medicinal purposes such as alexeteric, antihelminthic, antiurolithiatic, anti-inflammatory and diuretic properties. So, the present study has been made to find the phytochemicals and the properties that are exhibited by the different extracts of the *Sesbania aculeata*.

2. Materials and Methods

2.1. Plant Materials and Preparation of Extracts

Different plant parts (stem, roots, seeds and seeds coat) of *S. aculeata* were collected, cleaned, washed, shade dried and powdered coarsely. The extraction was performed using 15 g powdered plant parts in a Soxhlet apparatus using different solvents (water, ethanol, acetone, diethyl ether) at their boiling temperature. The other extraction conditions were the same as the ones that had been previously optimized [13]. The extracts obtained in the form of powder (concentrated form) were weighed for calculating the extraction yield and stored at 4°C until further use.

2.2. Quantitative Estimation of Phytochemicals

The obtained extracts were analyzed by means of quantitative phytochemical screening following the standard protocols [6, 11, 15, 21, 22].

2.3. In Vitro Antioxidant Activities

The antioxidant potential of the extracts was checked with DPPH free radical scavenging and ferric reducing power methods [4, 10]. For the radical scavenging activity, 2 ml of DPPH (0.1 mM) were added to 2 ml extract (20-100 µg/ml) followed by incubation in the dark at RT for 30 min. Absorbance measured at 517 nm with ascorbic acid as control. Low absorbance indicated higher antioxidant activity expressed as DPPH radical inhibition percentage. Reducing activity was checked by adding 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide (1%) to 2 ml extract (20-100 µg/ml) followed by incubation at 50°C for 20 min. The reaction was terminated by adding 1 ml TCA (10%) and then centrifuged at 3000 rpm for 15 minutes. After that ferric chloride

(0.1%) was added to the supernatant and absorbance was taken at 700 nm with ascorbic acid as control. The reducing power of all the extracts was calculated.

2.4. Anticancer Activity

The anticancer activity of extracts was checked by following the method of Skehan et al. [23] at ACTREC, Mumbai against human kidney cancer cell line 786-O and human oral cancer cell line SSC-29B for the ethanolic extracts. The cells were inoculated in 96 well titre plates and the in-situ fixation was done by using TCA (trichloro acetic acid). Solubilised extracts were added to cell suspension to get the final concentration of 10, 20, 40 and 80 µg/ml. Adriamycin (anticancer drug) was used as positive control. Fixed cells were stained with a Sulforhodomine B solution and the end point measurement was done for result interpretation at a reference wavelength of 690 nm and absorbance was noted at 540 nm. The percentage of growth inhibition was calculated as:

$$GI\left[\%\right] = \frac{T_i - T_z}{C - T_z} \cdot 100 \qquad (1)$$

where:
Gi is the growth inhibition [%];
Ti – the test growth;
Tz – the growth at time zero;
C – the control growth.

Experimental data of cell viability against extract concentration was estimated by using the linear regression method.

3. Results and Discussion

3.1. Extraction Yield

The percentage yield of crude extracts of different parts was checked for different solvents. The highest yield obtained for the aqueous extracts of stem was 26.40% which was followed by seeds (i.e. 21.10%). Diethyl ether yielded the lowest percentage yield for all the plant parts used (i.e. 5.75-9.48%). The highest yield for crude extracts of roots and seed coat was 19.10 and 24.35% respectively, obtained for ethanolic extraction (Table 1). Differences in the yield may be because of the differences in the polarity of the solvents used which resulted in the differential extraction of the various phytochemicals present in the plant parts [17].

3.2. Phytochemical Quantification

The quantity of the various phytochemicals tested varies greatly with the plant parts used and also among parts according to the solvent used for the extraction (Table 2). The alkaloid content was found higher in the alcoholic extract whereas it was lower in the aqueous extracts. The highest amount of alkaloid was found in the ethanolic extracts of stem (9.45 mg/g) while the lowest was found in the aqueous extracts of seeds (1.38 mg/g).

The amount of alkaloids concentration varies according to the solvent used, in the pattern of ethanol > acetone > diethyl ether > water. Whereas, the concentration of phenols, tannins, saponins and

flavonoids varied with different patterns of solvent used with the highest detected concentration in ethanolic extracts while the lowest was found in diethyl ether extracts irrespective of the plant part studied. The concentration of the phytochemicals in the acetone and aqueous extracts varies according to the plant part used. The highest amount of all the tested phytochemicals that are phenols, tannins, saponins and flavonoids were obtained in ethanolic extracts of seed coat.

Table 1

Percentage [%] yield of different solvent extracts of different parts of Sesbania aculeata

Plant part → / Solvent used ↓	Roots	Stem	Seeds	Seed coat
Water	14.20	26.40	21.10	13.60
Ethanol	19.10	21.00	20.55	24.35
Acetone	13.00	14.80	17.35	18.55
Diethyl ether	5.75	8.40	9.48	8.15

Among the plant parts tested, the highest phenols were obtained in seed coat followed by roots, stem and seeds for all the solvents used. The presence of phenol is thought to be responsible for the antibacterial and anti-inflammatory properties of the plants [1, 16]. The highest tannins were obtained in seed coat followed by stem, seeds and roots in all solvents except diethyl ether where seeds recorded the lowest. The saponin concentration exhibited significant variations depending on the plant parts and the solvent employed for the extraction procedure, with the lowest concentration observed in the diethyl ether extract of stem (3.55 mg/g). Likewise, the flavonoids concentration also showed variations among the plant parts and solvents used for extraction with the lowest concentration reported for the diethyl ether extract of roots (9.68 mg/g). Flavonoids are poly-phenolic compounds and are known to have various health benefit properties such as anti-oxidant, anti-inflammatory, anti-allergic and anti-cancer properties [8]. The variations in the concentrations of the phytochemicals among different solvents used are because of the degree of the polarity of the solvent used and the solubility of the compound [5]. These differences in the quantity of the phytochemicals are thought to be responsible for the diverse uses of the different parts of the *Sesbania* plants in Ayurveda [12].

Table 2

Alkaloids, phenols, tannins, saponins and flavonoids content of different solvent extracts of different parts of Sesbania aculeata

Phytochemical		Alkaloids [mg/g]	Phenols [g GAE/g	Tannins [mg GAE/g]	Saponins [mg DE/g]	Flavonoids [mg RE/g]
Extract						
Roots	Water	1.52±0.04	12.43±0.24	9.56±0.16	7.25±0.19	14.55±0.41
	Ethanol	8.34±0.21	22.76±0.31	14.80±0.32	11.35±0.27	19.16±0.19
	Acetone	7.42±0.32	14.85±0.54	12.90±0.43	5.78±0.22	15.35±0.34
	Diethyl ether	2.85±0.19	7.95±0.17	2.23±0.09	4.56±0.05	9.68±0.15
Stem	Water	1.43±0.07	12.23±0.19	11.45±0.22	6.87±0.43	13.90±0.25
	Ethanol	9.45±0.43	19.95±0.24	15.68±0.12	13.24±0.19	18.72±0.22
	Acetone	8.40±0.23	13.65±0.43	11.57±0.33	4.35±0.23	14.45±0.31
	Diethyl ether	3.05±0.09	6.34±0.29	2.98±0.11	3.55±0.33	10.68±0.19
Seeds	Water	1.38±0.05	11.24±0.22	10.65±0.41	7.42±0.31	12.87±0.24
	Ethanol	8.95±0.24	18.55±0.33	12.44±0.37	12.84±0.40	15.25±0.34
	Acetone	6.55±0.54	10.45±0.12	10.66±0.23	5.76±0.29	14.66±0.22
	Diethyl ether	2.87±0.13	5.38±0.09	1.87±0.07	4.65±0.18	12.80±0.11
Seed coat	Water	1.67±0.09	13.65±0.32	13.86±0.17	8.44±0.34	15.75±0.29
	Ethanol	7.90±0.41	29.20±0.18	15.90±0.28	14.15±0.22	21.46±0.12
	Acetone	6.75±0.36	16.76±0.44	14.45±0.12	7.15±0.15	16.13±0.23
	Diethyl ether	4.88±0.13	8.15±0.23	3.36±0.08	6.28±0.09	11.08±0.21

3.3. Antibacterial Activity

The different extracts of the different parts of *Sesbania aculeata* checked for antibacterial activity and all found to exhibit potential against the tested bacterial strain though it varied greatly with the parts and solvent used for extraction (Table 3). Best antibacterial response was exhibited by the ethanolic extracts for all the tested parts with a zone of inhibition ranging 20.8-31.1 mm in diameter which was close to the standard drug, namely cefotaxime used for the study (29.3-34.1 mm diameter). The least activity was reported in the diethyl ether extracts for all the parts tested with a zone of inhibition which ranged 5.7-12.7 mm in diameter. These differences in the antibacterial potential for different solvents may be because of differences in the content of the phytochemicals reported which are thought to be responsible for such activity. Diethyl ether showed the lowest yield and concentration of most phytochemicals which may be responsible for its extract's lowest

antibacterial activity.

Table 3

*Antibacterial activity of different extracts of S. aculeata against different bacteria**

		S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*B. subtilis*
Cefotaxime		29.5±0.46	34.1±0.31	32.7±0.12	33.4±0.22	29.3±0.19
Roots	Water	13.2±0.12	11.3±0.32	9.89±0.19	10.53±0.13	8.47±0.33
	Ethanol	23.7±0.34	25.3±0.41	26.1±0.24	24.8±0.21	23.6±0.15
	Acetone	21.6±0.75	23.4±0.16	22.1±0.10	22.1±0.40	21.6±0.13
	Diethyl ether	09.3±0.61	07.2±0.19	06.4±0.25	06.5±0.22	05.7±0.25
Stem	Water	14.9±0.19	13.1±0.27	12.2±0.33	12.3±0.28	11.6±0.33
	Ethanol	22.9±0.23	23.3±0.35	22.7±0.39	22.1±0.11	21.8±0.27
	Acetone	20.4±0.43	21.1±0.06	20.3±0.44	20.8±0.33	19.9±0.44
	Diethyl ether	10.1±0.09	10.7±0.18	10.0±0.16	10.3±0.51	09.8±0.39
Seeds	Water	12.9±0.14	12.9±0.49	13.4±0.73	13.1±0.48	12.8±0.55
	Ethanol	20.8±0.41	21.5±0.42	23.6±0.51	21.7±0.12	20.9±0.31
	Acetone	18.7±0.64	18.9±0.36	19.0±0.11	19.0±0.19	18.7±0.43
	Diethyl ether	09.8±0.19	09.9±0.24	10.1±0.46	10.0±0.37	09.7±0.14
Seed coat	Water	15.3±0.44	15.1±0.22	15.8±0.45	15.7±0.27	15.1±0.46
	Ethanol	27.2±0.34	31.1±0.43	29.7±0.08	29.8±0.25	27.1±0.29
	Acetone	23.7±0.11	25.3±0.54	24.9±0.27	25.0±0.34	23.5±0.23
	Diethyl ether	12.5±0.54	11.9±0.44	11.3±0.24	11.1±0.23	10.1±0.36
*Zone of inhibition in 'mm'						

3.4. *In Vitro* Antioxidant Activity

The presence of phenols and flavonoids is thought to be responsible for the antioxidant potential that is contributed to by the hydroxyl groups present [2]. Most widely used method for checking the antioxidant potential is the DPPH free radical scavenging activity that uses DPPH which is an artificial organic free radical [7]. All the extracts showed free radical scavenging activity as tested by the DPPH free radical scavenging activity although with significant differences among the solvents used. The ethanolic extract showed the highest antioxidant activity, followed by acetone, water and diethyl ether extracts showing the lowest activity for all the plant parts tested (Figures 1 to 4).

The antioxidant activity increased with the increasing concentration of the extracts. Among the plant's parts studied, the ethanolic seed coat extract showed the highest activity of 73% scavenging activity which was found close to the ascorbic acid, control (81%) at 100 µg/ml concentration whereas diethyl ether seed coat extract showed only 36% scavenging activity at the same concentration.

Similar results obtained for the ferric reducing activity where the highest activity which was obtained for ethanolic extracts. These results correspond with the content of phytochemicals particularly flavonoids and phenols which are thought to be responsible for the antioxidant potential. Although the activity of the extracts is less than the control used, which may be due to the fact that the control is pure compound whereas extracts are a crude mixture of various compounds. Natural antioxidant flavones glycoside hasalso been previously reported from the stems of *S. aculeata* [20] which showed good antioxidant potential. The results are in accordance with the previous reports in other legumes, where alcoholic extracts showed good antioxidant potential [14, 19].

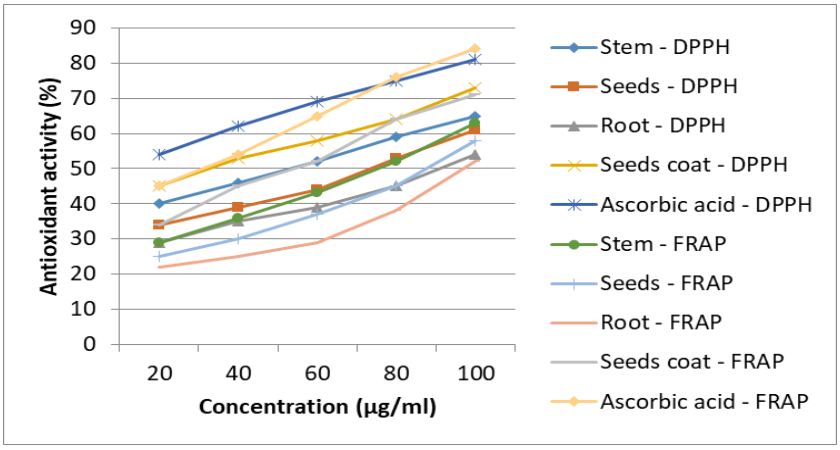

Fig. 1. *Antioxidant activity of ethanolic extracts of different parts of S. aculeata at different concentration*

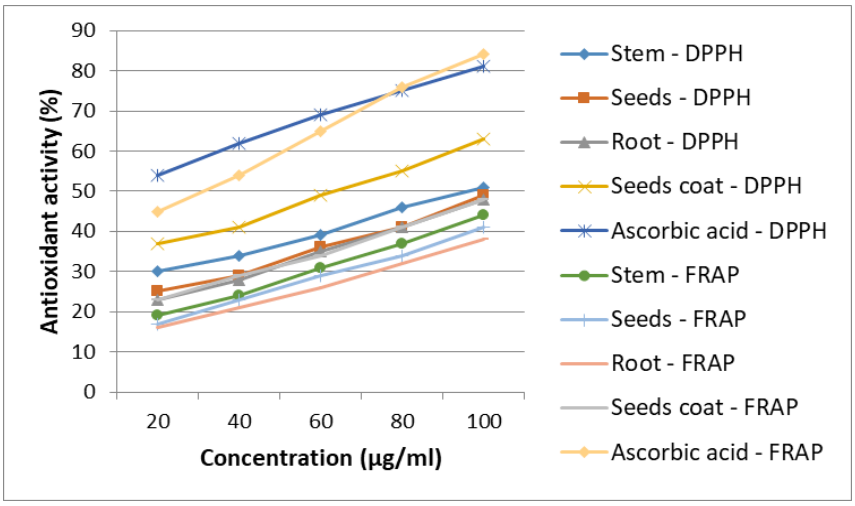

Fig. 2. *Antioxidant activity of acetone extracts of different parts of S. aculeata at different concentration*

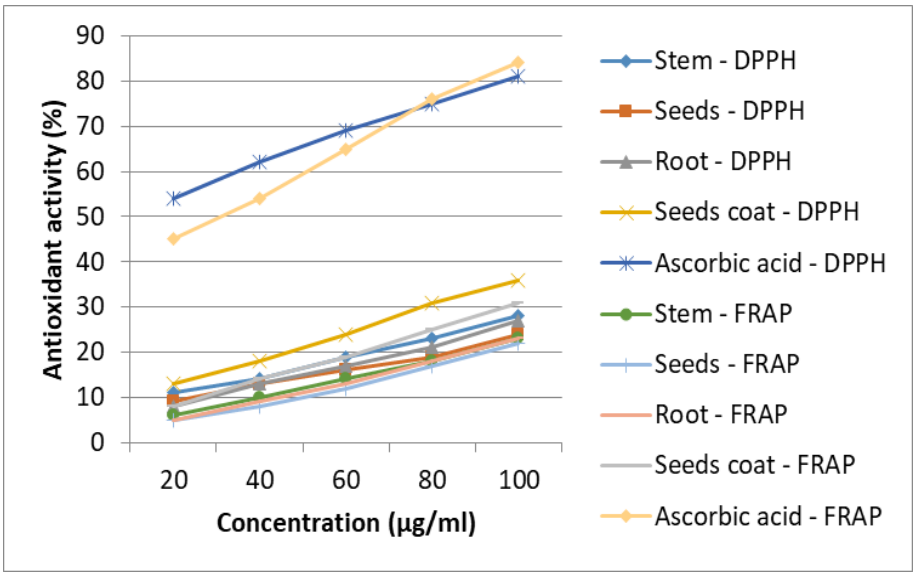

Fig. 3. *Antioxidant activity of acetone extracts of different parts of S. aculeata at different concentration*

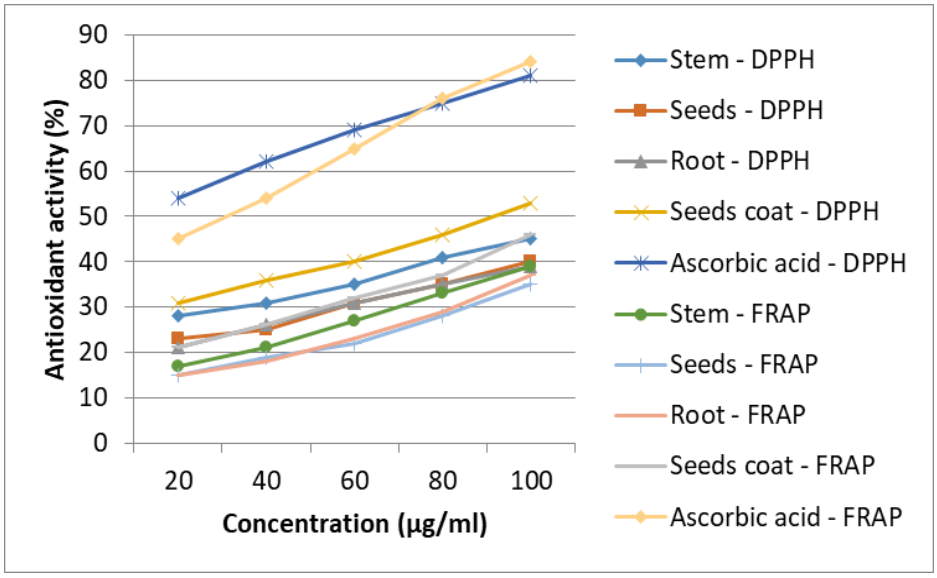

Fig. 4. *Antioxidant activity of diethyl ether extracts of different parts of S. aculeata at different concentration*

3.5. Anticancer Activity

The first step towards the search for the new anticancer metabolites is the evaluation of the cytotoxicity. So, in the present study, the *in vitro* cytotoxicity has been checked by SRB assay against two different cancer cell lines, human kidney cancer cell line 786-O and human oral cancer cell line SSC-29B for the

ethanolic extracts. Only seed coat extract showed some activity against the tested lines. Seed coat extracts controlled the growth of the cancer cells by 68 and 64% against the human oral cancer cell line SSC-29B and human kidney cancer cell line 786-O, respectively (Figures 5 and 6).

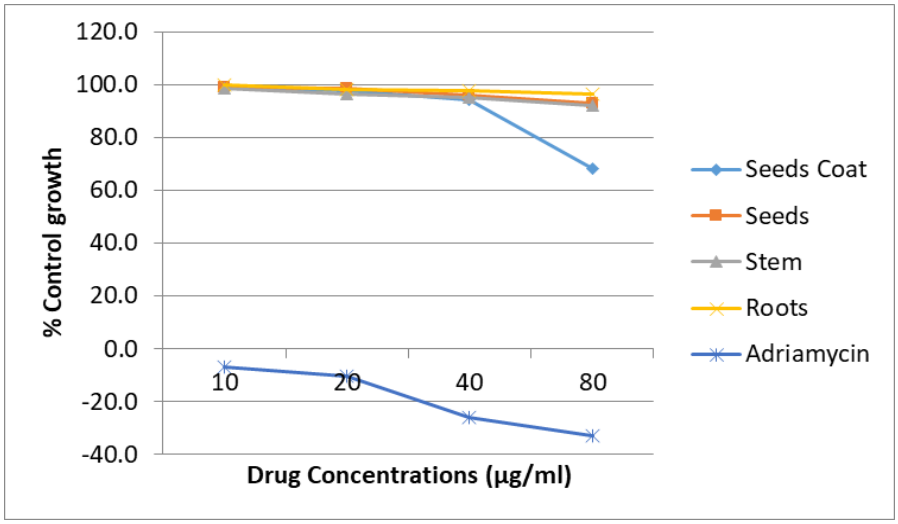

Fig. 5. *Effect of ethanolic extracts of different parts S. aculeata on growth of human oral cancer cell line SSC-29B*

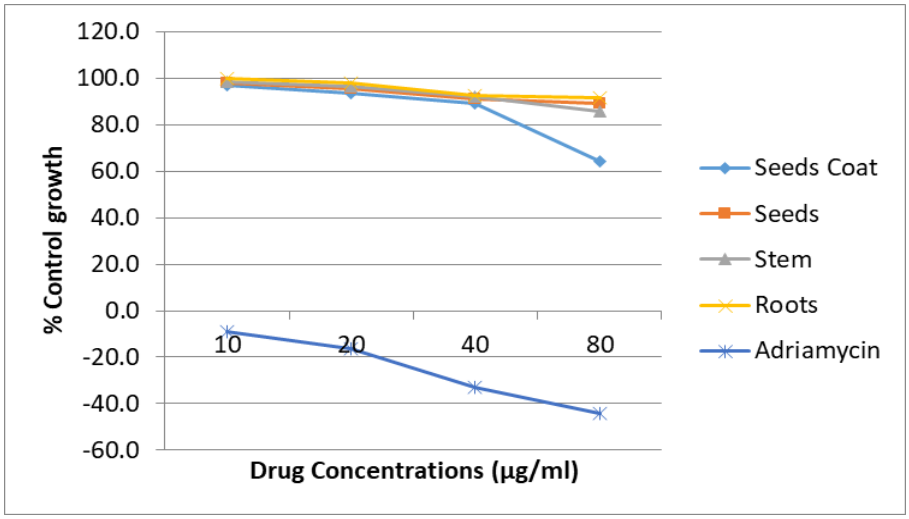

Fig. 6. *Effect of ethanolic extracts of different parts S. aculeata on growth of human kidney cancer cell line 786-O*

Although the activity of the plant extracts was considerably inferior to the control drug (Adriamycin) used, which may be determined by the fact that the extract is a mixture of compounds whereas the drug is a pure compound. The results are in accordance with the previous studies in other legumes where

seed coat extracts showed some activity against the tested cell lines [14, 19]. In other species of the *Sesbania* genus, extracts of the *Sesbania grandiflora* have shown anticancer activity against the neuroblastima cell line [18] and colon cancer cell line [9] when tested *in vitro* and both *in vivo* and *in vitro* respectively.

4. Conclusions

So, it can be concluded that the extracts of *Sesbania aculeata* can be used as a potential source of various phytochemicals which are source of antibacterial, antioxidant and anticancer properties. Ethanolic seed coat extracts in particular, which have shown good anticancer activity against the tested cancer lines should be further explored for the active compound that is responsible for such activity as well as with other types of cancer cell lines, so that it could be used as control measures against various deadly dangerous diseases including cancer.

Acknowledgements

The author Nikhil Mehta is thankful to University Grant Commission (UGC), New Delhi, India for the award of Junior Research fellowship. Ritu Badal is thankful to University Grant Commission (UGC), New Delhi, India for the award of Rajiv Gandhi National Fellowship.

References

1. Adebayo, S.A., Dzoyem, J.P., Shai, L.J. et al., 2015. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. In: BMC Complementary and Alternative Medicine, vol. 15, ID article 159. DOI: 10.1186/s12906-015-0669-5.
2. Asha, K.K., Suseela, M. Lakshmanan, P.T., 2012. Flavonoids and phenolic compounds in two mangrove species and their antioxidant property. In: Indian Journal of Marine Sciences, vol. 41(3), pp. 259-264.
3. Duke, J.A., 1981. Handbook of legumes of world economic importance. In: Plenum Press, New York, pp. 216-217. DOI: 10.1007/978-1-4684-8151-8.
4. Elansary, H.O., Salem, M.Z.M., Ashmawy, N.A. et al., 2012. Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt. In: Journal of Agricultural Sciences, vol. 4(10), pp. 144-152. DOI: 10.5539/jas.v4n10p144.
5. Ghasemzadeh, A., Jaafar, H.Z., Rahmat, A., 2011. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. In: Journal of Medicinal Plant Research, vol. 5(7), pp. 1147-1154.
6. Jia, Z., Tang, M., Wu, J., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. In: Food Chemistry, vol. 64(4), pp. 555-559. DOI: 10.1016/S0308-8146(98)00102-2.
7. Kedare, S.B., Singh, R.P., 2011. Genesis and development of DPPH method of antioxidant assay. In: Journal of Food Science and Technology, vol. 48, pp. 412-422.

DOI: 10.1007/s13197-011-0251-1.
8. Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. In: Scientific World Journal, vol. 2013, ID article 162750. DOI: 10.1155/2013/162750.
9. Laladhas, K.P., Cheriyan, V.T., Puliappadamba, V.T. et al., 2010. A novel protein fraction from *Sesbania grandiflora* shows potential anticancer and chemopreventive efficacy, in vitro and in vivo. In: Journal of Cellular and Molecular Medicine, vol. 14, pp. 636-646. DOI: 10.1111/j.1582-4934.2008.00648.x.
10. Loo, A.Y., Jain, K., Darah, I., 2007. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. In: Food Chemistry, vol. 104(1), pp. 300-307. DOI: 10.1016/j.foodchem.2006.11.048.
11. Makkar, H.P., Siddhuraju, P., Becker, K., 2007. Methods in molecular biology: plant secondary metabolites. Human Press, Totowa, New Jersey, U.S.A. DOI: 10.1007/978-1-59745-425-4.
12. Malviya, R., Sharma, R., Mishra, A. et al., 2013. Agasthya (*Sesbania grandiflora* Linn.): Ayurvedic approach. In: Universal Journal of Pharmacy, vol. 2(4), pp. 1-5.
13. Mehta, N., Rao, P., Saini, R., 2018. Efficient extraction of thermostable metabolites of *Sesbania aculeata* and *in vitro* evaluation for their antibacterial potential. In: Analele Universitatii din Oradea, Fascicula Biologie, vol. 25(1), pp. 33-41.
14. Mehta, N., Rao, P., Saini, R., 2021. Exploration of the antibacterial, antioxidant and anticancer potential of the seed coat extract of mungbean (*Vigna radiata* L. Wilczek). In: Plant Achieves, vol. 21(1), pp. 1628-1633. DOI: 10.51470/PLANTARCHIVES.2021.v21.no1.222.
15. Obadoni, B.O., Ochuko, P.O., 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. In: Global Journal of Pure and Applied Science, vol. 8(2), pp. 203-208. DOI: 10.4314/gjpas.v8i2.16033.
16. Ouattara, M.B., Konaté, K., Kiendrébéogo, M. et al., 2011. Antibacterial potential and antioxidant activity of polyphenols of *Sesbania grandiflora*. In: Current Research Journal of Biological Science, vol. 3(4), pp. 351-356.
17. Paulsamy, S., Jeeshna, M.V., 2011. Preliminary phytochemistry and antimicrobial studies of an endangered medicinal herb *Exacum bicolor* Roxb. In: Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 2(4), pp. 447-457.
18. Ponnanikajamideen, M., Nagalingam, M., Vanaja, M. et al., 2015. Anticancer activity of different solvent extracts of *Sesbania grandiflora* against neuroblastima (IMR-32) and colon (HT-29) cell lines. In: European Journal of Biomedical and Pharmaceutical Sciences, vol. 2(3), pp. 2349-8870.
19. Rao, P., Mehta, N., Saini, R., 2019. Exploration of medicinal properties of *Macrotyloma uniflorum* – an important grain legume crop plant. In: Analele Universitatii din Oradea, Fascicula Biologie, vol. 26(1), pp. 27-33.

20. Satnami, D.K., Yadava, R.N., 2012. Natural antioxidant (flavone glycoside) from *Sesbania aculeata* Pers. and its potential activity. In: International Journal of Pharmaceutical Sciences and Research, vol. 3(8), pp. 2819-2824. DOI: 10.13040/IJPSR.0975-8232.3(8).2819-24.
21. Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. In: Journal of Agricultural and Food Chemistry, vol. 51(8), pp. 2144-2155. DOI: 10.1021/jf020444+.
22. Siddhuraju, P., Manian, S., 2007. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. In: Food Chemistry, vol. 105(3), pp. 950-958. DOI: 10.1016/j.foodchem.2007.04.040.
23. Skehan, P., Storeng, R., Scudiero, D. et al., 1990. New colorimetric cytotoxicity assay for anticancer drug screening. In: Journal of the National Cancer Institute, vol. 82(13), pp. 1107-1112. DOI: 10.1093/jnci/82.13.1107.